

Applicants: Michael Gallo et al.

ABOX-2 CIP CPA

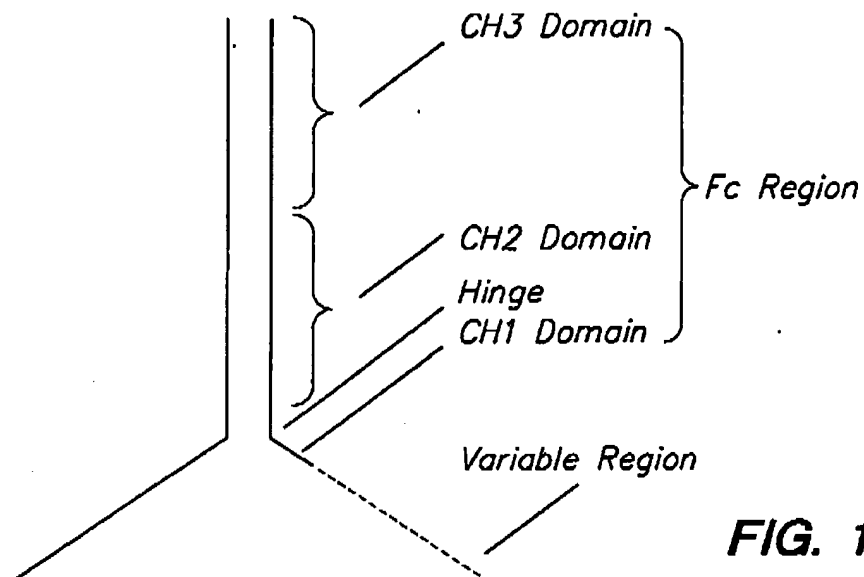
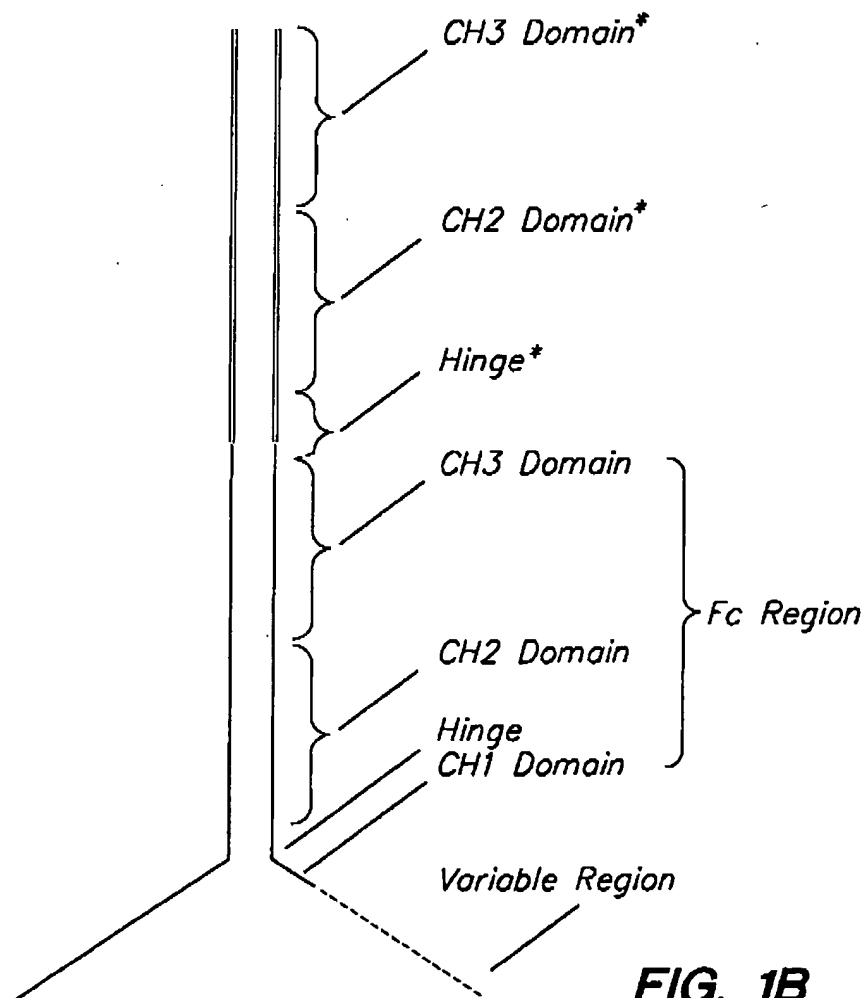
Application No.: 09/375,924

Filed: August 17, 1999

For: GENERATION OF MODIFIED MOLECULES
WITH INCREASED SERUM HALF-LIVES

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**FIG. 1A****FIG. 1B**

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STEP 1: CREATION OF UNIQUE Bsu36I RESTRICTION (CCTTAGG) SITE IN 3' TERMINUS OF GAMMA-4 CONSTANT REGION:

SEQUENCE ENCODING LAST 4 AMINO ACIDS OF HUMAN GAMMA-4 CONSTANT REGION:

TCT CTG GGT AAA [SEQ ID NO.:5]

MODIFIED SEQUENCE ENCODING THE SAME AMINO ACIDS:

TCC TTA GGG AAG [SEQ ID NO.:6]

STEP 2: PCR PRIMERS UTILIZED FOR ACCOMPLISHING SUCH MODIFICATION:

PRIMER 1:

5'γ4 OLIGO GGG ACC CAC GGG GTG CGA GGG C (Dra III) [SEQ ID NO.:7]

PRIMER 2:

3'γ4 OLIGO CTT CCC TAA GGA CAT GGA GAG GCT CTT CTG TGT GTG (Bsu36I) [SEQ ID NO.:8]

PRIMER 3:

5'γ1 OLIGO GAT TCC TTA GGG AAG GCA GAG CCC AAA TCT AGT GAC (Bsu36I) [SEQ ID NO.:9]
ser

PRIMER 4:

3'γ1 OLIGO GCC GGA ATT CGG TAC GTG CCA AGC ATC CTC GTG C (EcoR I) [SEQ ID NO.:10]

STEP 3: THREE WAY LIGATION:

- INTRODUCE NEW Bsu36I SITE AT GAMMA 4-HINGE JUNCTION
- ADD HINGE AND GAMMA 1 CH2 AND CH3 DOMAINS
- CLONE INTO DraIII-EcoRI SITES OF EXPRESSION VECTOR (VDJ-IgG4)

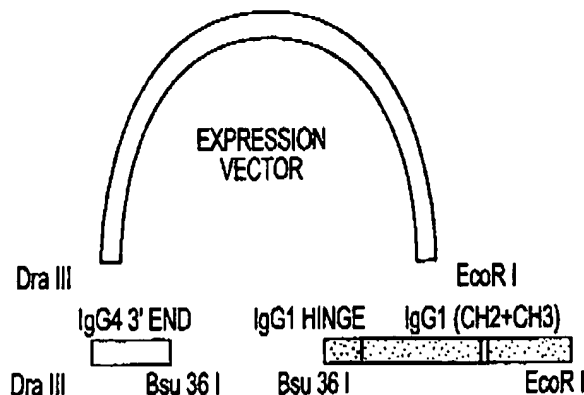


FIG. 2

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*Competition for binding with
protein AHRP*

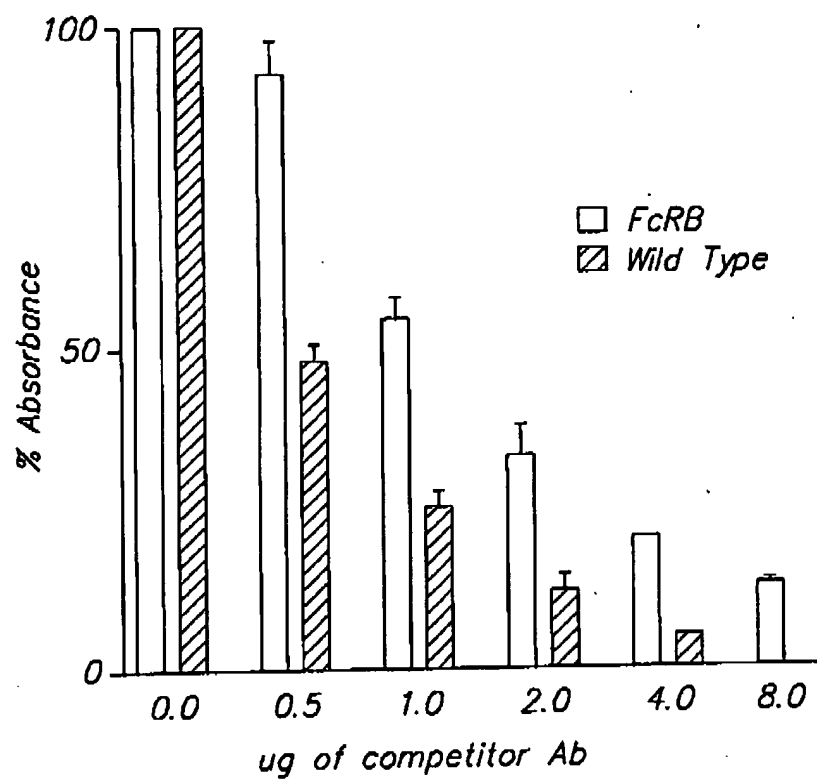


FIG. 3